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AN APPARATUS FOR THE MEASUREMENT OF PULMONARY FUNCTION IN UNANE--ETC(U)
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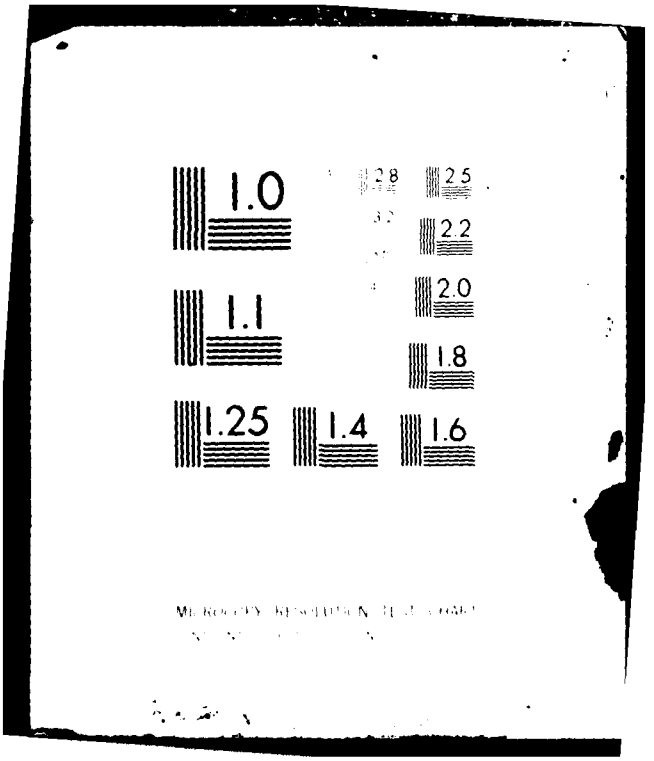
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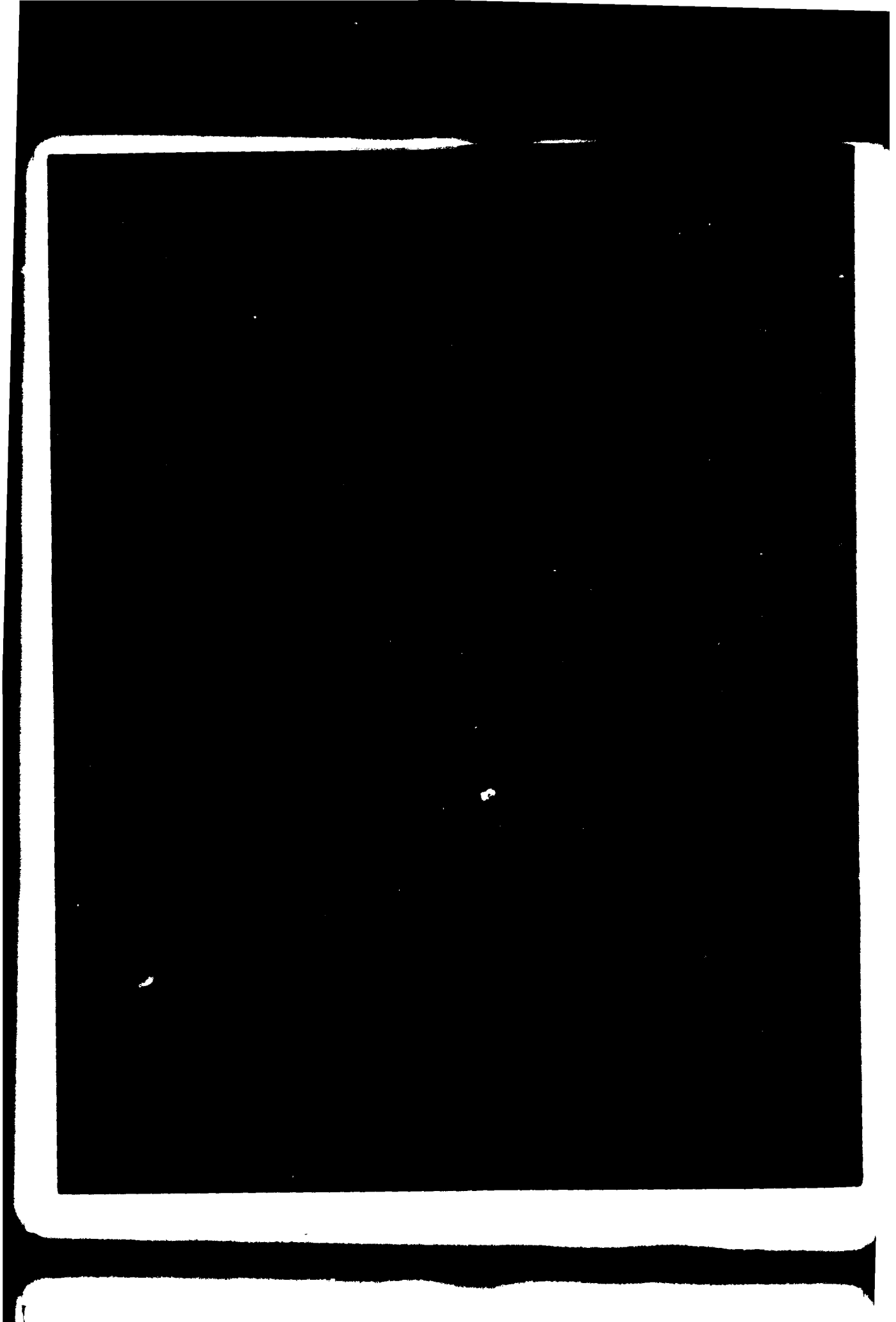

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White rat	Respiratory volumes	

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Because of public and political opinion, rising costs, and regulations, the use of some types of laboratory animals has been limited. As a result, small mammals – in particular, white rats – have become an acceptable alternative to canines for use in toxicological testing. In this report, a method is described by which unanesthetized and comfortably restrained rats are exposed by inhalation to yield fairly complete pulmonary information.

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PREFACE

The work described in this report was authorized under Project 1L162706A553, CB Defense and General Investigations. This work was started in June 1979 and completed in June 1980. The experimental data are contained in notebook 10,131.

In conducting the work described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

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AN APPARATUS FOR THE MEASUREMENT OF PULMONARY FUNCTION IN UNANESTHETIZED SMALL ANIMALS

1. INTRODUCTION

In many toxicological studies, substances are administered by the inhalation route resulting in the lung becoming the prime target organ. Therefore, it is necessary to include studies on pulmonary function as a standard toxicological assessment to measure bronchoconstrictive and fibrotic effects in short- and long-term exposures. In recent years, various studies have been underway to evaluate the different phases of pulmonary function in the small animal. This paper describes a method, developed in these laboratories, to test the effects of different airborne chemicals on the lung of the guinea pig or rat or both.

This technique involves the modification of the whole body plethysmograph used to assess pulmonary function in man,[1] infants,[2] and, more recently, small animals.[3-6] This system is currently being employed to test the effects of various substances on the lungs of rats and guinea pigs following acute, subacute, and chronic exposures.

2. PRINCIPLE OF OPERATION

2.1 Theory.

The operation of the whole body plethysmograph is based on the pressure changes that occur in the sealed box during the animal's respiratory cycle. As the animal inhales by expanding the chest, a positive pressure is produced in the box; on a normal exhalation, which is due to recoil of the lungs to residual volumes, the pressure in the box decreases or returns to end expiration volume. These pressure changes in the plethysmograph are considered to be inversely proportional to the pressure changes taking place in the lung of the animal.[1]

2.2 Human Application.

In the human body plethysmograph,[2] alveolar pressure (PA) is estimated by measuring pressure at the airway (mouth) opening. The ratio between airway pressure (PA) and pressure in the body plethysmograph (Pp) is determined while the subject's airway is completely obstructed for a short time (PA/Pp). Then the relationship between plethysmograph pressure (Pp) and pulmonary airflow (V) is measured while the subject pants at an approximate frequency of 85 breaths/minute through an open airway extension. Airway resistance is then calculated from the ratio of the obstructed and unobstructed slopes.

$$\frac{Pa/Pp}{V/Pp}$$

and has the units of cm H_2O/ℓ/second.

The rat plethysmograph functions similarly. The airway is obstructed and pressure measurements are taken. When the airway is opened, the normal respiratory pattern of the rat resembles panting in its frequency and the volumes are relatively constant.

2.3 Animal Application.

In this system, the respiratory flow rate (V) of the animal is being measured simultaneously with the plethysmograph pressure (P_1) changes. A small pneumotachometer is used to measure respiratory flow. Therefore, given the respiratory flow and the plethysmographic pressure (alveolar pressure) the total resistance can be estimated.

In order to better equate the lung pressure with the plethysmographic pressure, the respiratory pattern of the animal is interrupted and the pressure as close to the mouth as possible, as well as the plethysmographic pressure, is measured. Combining these data results in a factor that is incorporated into the calculation of the estimated pulmonary resistance. This calculation will be discussed later.

3. DESCRIPTION OF THE APPARATUS

3.1 Plethysmographic Box.

The plethysmographic box is constructed of clear 1/4-inch Plexiglas. The inside measurements are 12.6 cm high by 15.2 cm wide by 40.6 cm long, which constitutes a volume of 7776 ml. Holes are drilled in the sides of the box to accommodate hypodermic needles (16 gauge) for the recording of plethysmographic pressures and the differential pressures from the pneumotachometer. A hole is also located in the front of the box for the insertion of the interrupter plunger. A drawing of the box is shown in figure 1.

3.2 Animal Head Holder.

The animal headpiece is shown in figure 1. It is constructed of 1/8-inch Plexiglas and is roughly shaped to the animal head size. The front contains a port through which the pneumotachometer is connected. Between the holder and the restrainer, a stiff rubber collar is attached through which the animal's head is inserted; this collar helps to seal the animal's head from the trunk of the body, which allows all air from the respiration of the animal to pass through the pneumotachometer. The headpiece and restrainer are held together with screws and wing nuts. It is not necessary to disassemble the apparatus during the course of testing several animals of similar size; it is necessary to change the rubber collar to accommodate animals of varying sizes.

3.3 Pneumotachometer.

The pneumotachometer used with this apparatus was fabricated in these laboratories by Dr. E. G. Cummings, since one was not available commercially that would measure the size and flow rates desired for these tests. It consists of two plastic disposable 1-ml syringes with their bases abutted to one another with two pieces of 300-mesh screen between to offer resistance to flow. Holes are drilled around each syringe entering into a manifold where pressure is measured on each side of the wire screen. All of this section is covered and sealed with a band around the outside. All potential leak areas are sealed with a plastic glue. A 16-gauge needle is inserted into each manifold section and connected to a differential pressure transducer for the determination of flow. The total length is 5.5 cm.

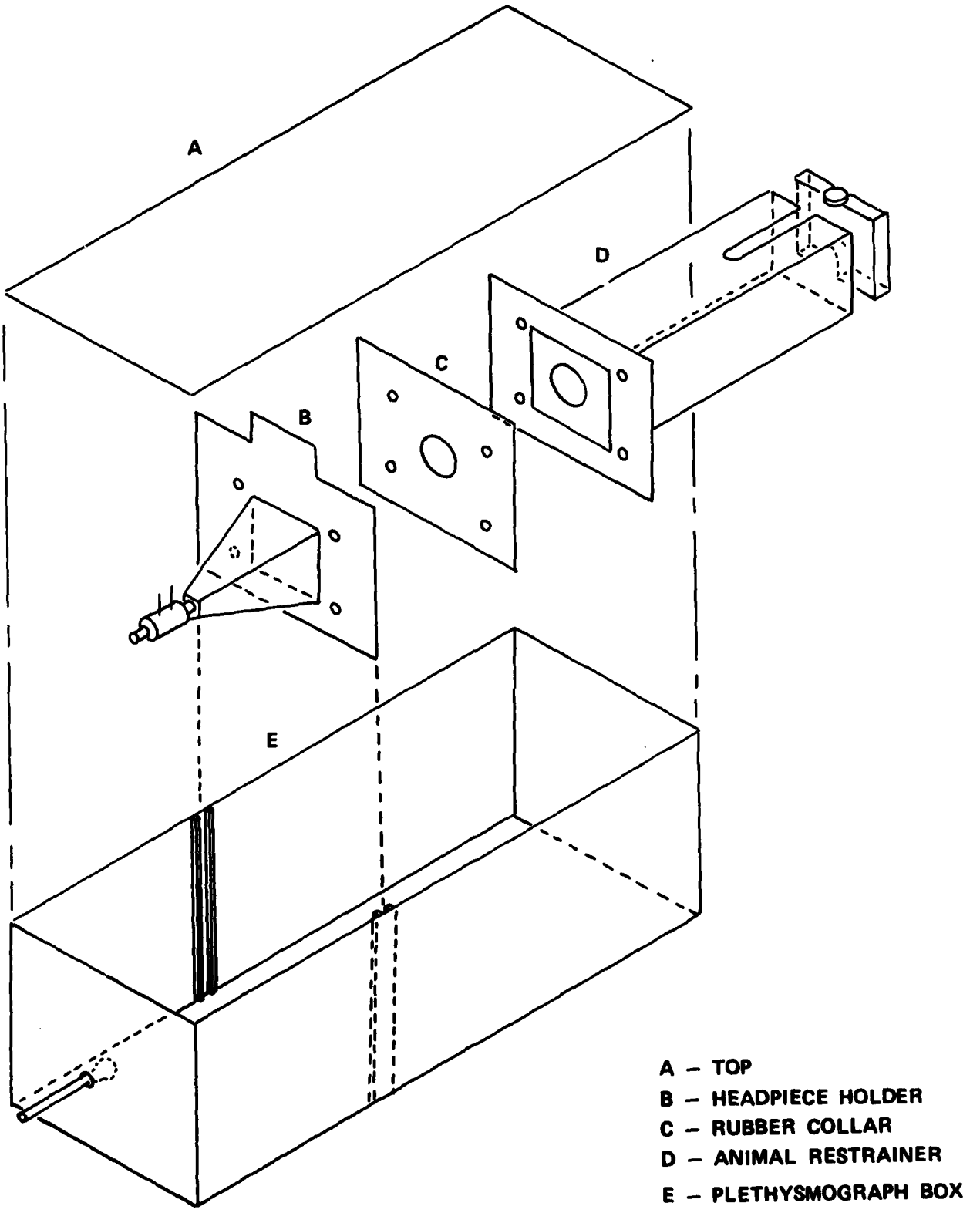


Figure 1. Small Animal Plethysmograph

4. CALIBRATION PROCEDURE

4.1 Equipment.

The equipment employed for this test includes two Hewlett-Packard Model 270 differential pressure transducers, whose signals are amplified and processed by a Series H-P 8800 amplifying and recording instrument. One transducer (flow) receives a small pressure signal from each side of the wire screen in the pneumotachometer. This transducer also is used to record pressures during the interruptive process which produce signals approximately 10 to 20 times those during normal pneumotachometer operation. During this phase, one side of the differential transducer is open to the atmospheric pressure. The second transducer is used to record the pressure changes in the plethysmographic box. The reference side of this transducer is always open to the atmosphere. Signals obtained from the flow transducer are recorded as flow in milliliters per second and the signal is integrated into volume to produce a record of tidal or minute volume, or both.

4.2 Calibration.

Both transducers are calibrated as pressure in millimeters of water. They are connected to a water manometer and varying pressures are applied to the transducer and recorded. Airflow is calibrated by drawing air through the pneumotachometer at varying rates as measured by a calibrated wet-test meter and rotometer and recording the deflection on the H-P 8800. The relationship of flow to deflection is linear within the ranges necessary for flow rates in small animals. Calibration of the tidal volume is accomplished in the same manner as the flow rate, integrating the signal received from the transducer. Further, tidal volume calibration is accomplished by using a spring-loaded syringe and injecting known volumes of air through the pneumotachometer and integrating the amplified signal. Formulas for each measurement have been developed from these curves and are used to calculate flows, pressures, and volumes from the records. All calibrations are checked before each test day and a complete recalibration is carried out about every 3 months.

5. TESTING PROCEDURE

The animal restrainer is attached to the headpiece with the rubber collar in place. The rat is placed in the restrainer with his head and ears through the rubber collar. Respiratory rate and flow are monitored immediately to determine the animal's state. Five to ten minutes in the apparatus are allowed for the animal to stabilize. After this period, the top is placed on the box and sealed. Time is allowed for pressures in the box to stabilize. Following stabilization, as determined by respiratory rate and volume, records are made of the respiratory flow and the plethysmographic pressure during normal respiratory cycles. During this time, the flow output is fed into a respiration integrator to record the tidal and minute volumes. During this procedure, the flow through the pneumotachometer is interrupted and the pressure changes in the headpiece and plethysmograph are measured. These values are used to relate the plethysmographic pressure to normal breathing effort, which in turn is employed in the calculation of the estimated pulmonary resistance. The animal is allowed to remain in the system for a 5- to 10-minute period; and respiratory rate, flow, and minute and tidal volumes are recorded. Following the testing procedure, the rat is removed from the apparatus and returned to its original cage.

6. DETERMINATION OF ESTIMATED PULMONARY RESISTANCE

Pulmonary resistance is determined by the pressure changes in the alveoli that will move air into and out of the lungs at a standard flow rate. It is, therefore, necessary to estimate the pressure in the alveoli during a respiratory cycle. Tests on humans and other animals have indicated that when the subject is placed in a sealed plethysmograph and allowed to breathe normally, the pressure changes in the plethysmograph are proportional to the pressure changes occurring in the lung.[4] Using this premise, resistance can be estimated in an animal if flow and plethysmographic pressures are known. In this instance, resistance may be estimated by relating plethysmographic pressure to respiratory flow in the following way:

$$\text{Estimated pulmonary resistance } (cmH_2O/\ell/sec) = \frac{\text{plethysmographic pressure } (P_1)\ (cmH_2O)}{\text{respiratory flow } (F_1)\ (\ell/sec)}$$

To better relate the plethysmographic pressure changes with pressure changes in the lungs in the animal, the interruption technique was added to this testing method. This involves measuring the pressure in the plethysmograph and, simultaneously, the pressure as close as possible to the mouth of the animal during the time that the flow through the pneumotachometer is interrupted. The relationship between the plethysmographic presssure and mouth pressure during a near maximum effort for the animal to move air will give a factor that can be applied to the previous formula that should give results nearer to the actual pulmonary resistance of the animal. This factor is calculated as follows:

$$\text{Factor} = \text{mouth pressure } (cmH_2O)\ (P_2)/\text{plethysmographic pressure } (cmH_2O)(P_3)$$

Applying this to the resistance measurement:

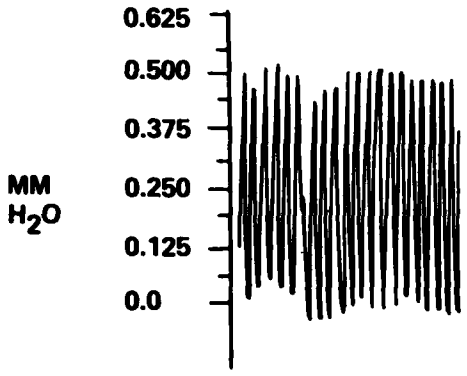
$$\text{Estimated pulmonary resistance} = (P_1/F_1) \times (P_2/B) = P_1P_2/P_3/F_1 = cmH_2O/\ell/sec$$

This relationship is essentially the same as described earlier for the human body plethysmograph.

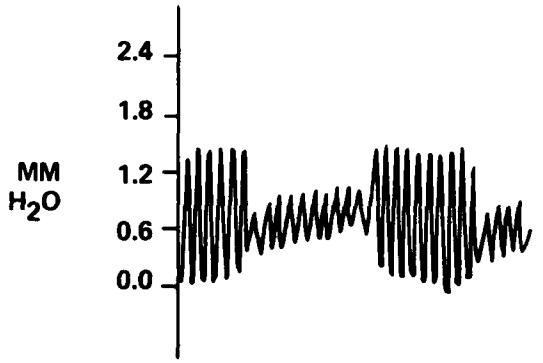
7. EXAMPLES OF RECORDS FROM A NORMAL RAT AND A RAT WITH HIGH PULMONARY RESISTANCE

Recordings were made from control animals which were considered to be normal in terms of pulmonary resistance and whose lungs were found to be pathologically negative from subsequent necropsy and histology. An example of this recording is shown in figure 2. Combined data extracted from these records are given in the table.

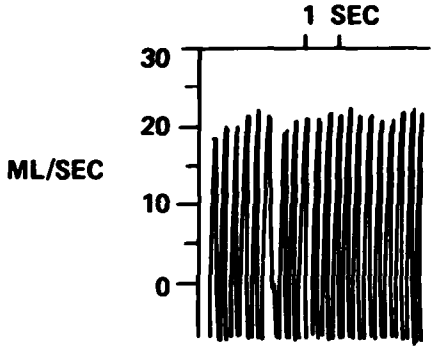
Recordings were also made from animals exposed to diesel fuel exhaust. In this group, a number of animals were found to have increased pulmonary resistance. A sample of such a recording is shown in figure 3.



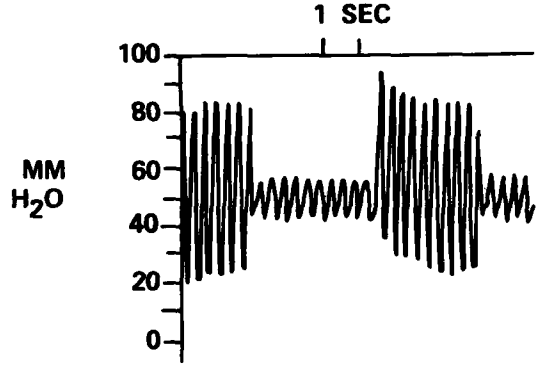
PLETHYSMOGRAPHIC PRESSURE
DURING NORMAL RESPIRATION (P_1)



PLETHYSMOGRAPH
DURING INTERRUPTION (P_3)



INSPIRATORY FLOW (F)



INTERRUPTED PRESSURE (P_2)

P_1 = 0.500 MM H_2O

P_2 = 60.0 MM H_2O

P_3 = 1.4 MM H_2O

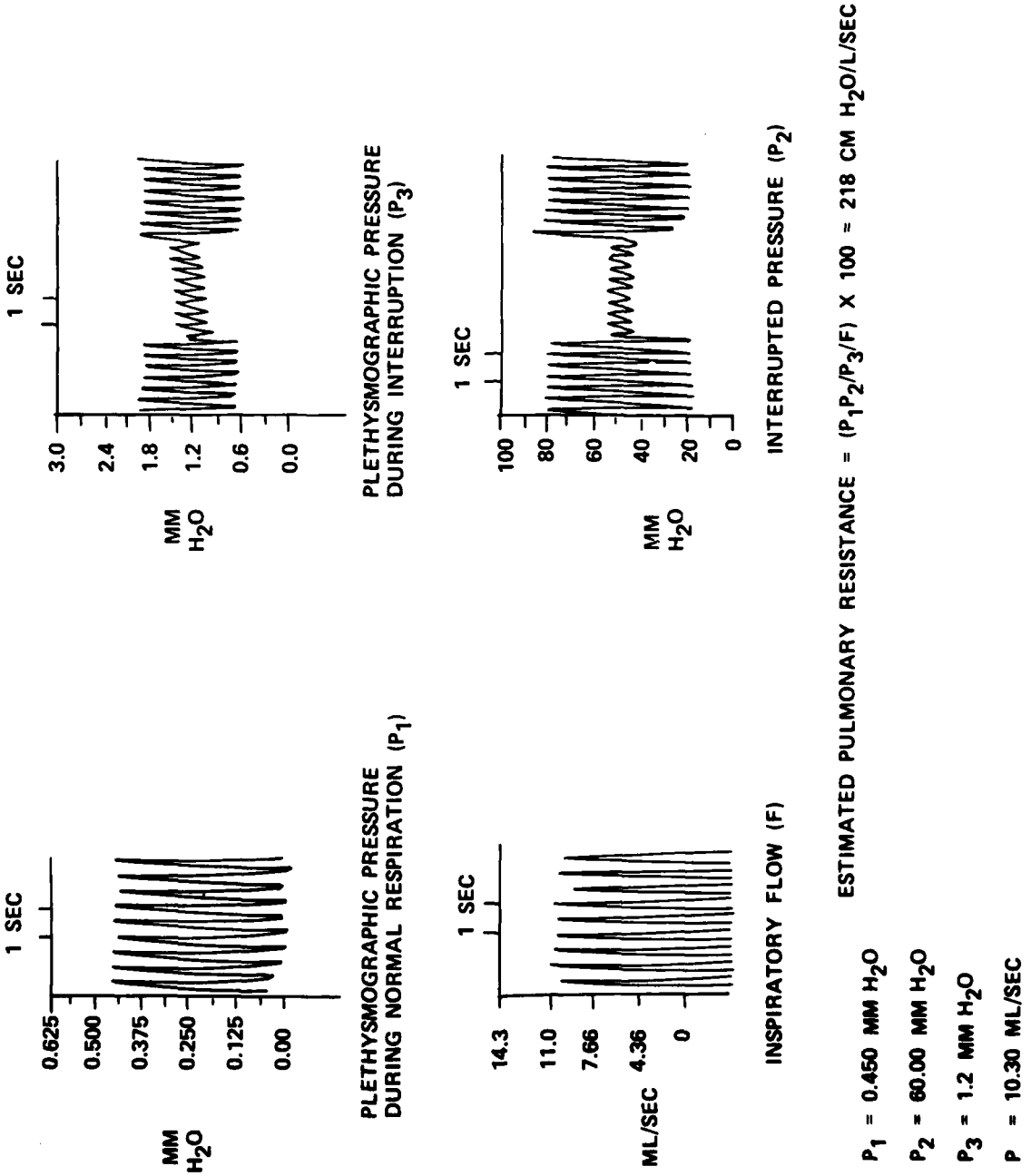
F = 19.0 ML/SEC

ESTIMATED PULMONARY RESISTANCE = $(P_1P_2/P_3/_F)$ X 100 = 112.8 CM H_2O/L/SEC

Figure 2. Control Plethysmograph Record

Table. Summary of the Estimated Pulmonary Resistance of Normal Rats as Determined in These Laboratories

Sex	Number	Mean ± S.E.	Range
		$cmH_2O/\ell/sec$	
Males	15	94.3 ± 6.3	68.1-149.8
Females	15	70.6 ± 4.1	52.1-117.9



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Wright-Patterson AFB, OH 45433

AMD/RDE
ATTN: LTC T. Kingery 1
Brooks AFB, TX 78235

OUTSIDE AGENCIES

Battelle, Columbus Laboratories
ATTN: TACTEC 1
505 King Avenue
Columbus, OH 43201

Toxicology Information Center,
WG 1008
National Research Council 1
2101 Constitution Ave., NW
Washington, DC 20418

US Public Health Service
Center for Disease Control
ATTN: Lewis Webb, Jr. 1
Building 4, Room 232
Atlanta, GA 30333

END

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